

Scotland's Rural College

Genetic dissection of complex behaviour traits in German Shepherd dogs

Friedrich, Juliane; Strandberg, Erling; Arvelius, Per; Sánchez-Molano, Enrique; Pong-Wong, Ricardo; Hickey, John; Haskell, MJ; Wiener, Pamela

Published in:
Heredity

DOI:
[10.1038/s41437-019-0275-2](https://doi.org/10.1038/s41437-019-0275-2)

Print publication: 14/10/2019

Document Version
Peer reviewed version

[Link to publication](#)

Citation for published version (APA):
Friedrich, J., Strandberg, E., Arvelius, P., Sánchez-Molano, E., Pong-Wong, R., Hickey, J., Haskell, MJ., & Wiener, P. (2019). Genetic dissection of complex behaviour traits in German Shepherd dogs. *Heredity*, 123(6), 746-758. <https://doi.org/10.1038/s41437-019-0275-2>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 Genetic dissection of complex behaviour traits in German Shepherd dogs

2 Juliane Friedrich¹, Erling Strandberg², Per Arvelius³, E. Sánchez-Molano¹, Ricardo Pong-
3 Wong¹, John M. Hickey¹, Marie J. Haskell^{4*}, Pamela Wiener^{1*}

4 ¹Division of Genetics and Genomics, The Roslin Institute and Royal (Dick) School of
5 Veterinary Studies, University of Edinburgh, Midlothian, EH25 9RG, UK

6 ²Department of Animal Breeding and Genetics, Swedish University of Agricultural
7 Sciences, PO Box 7023, S-750 07 Uppsala, Sweden

8 ³Swedish Armed Forces Dog Training Centre, Box 194, SE-195 24 MÄRSTA, Sweden

9 ⁴Animal and Veterinary Sciences Group, Scotland's Rural College, Edinburgh, EH25 9RG,
10 UK

11

12 *Corresponding authors

13 Pamela Wiener: Division of Genetics and Genomics, The Roslin Institute and
14 Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian,
15 EH25 9RG, UK; Telephone: +44 (0)131 651 9100; Fax: +44 (0) 131 651 9105;
16 pam.wiener@roslin.ed.ac.uk

17 Marie Haskell: Animal and Veterinary Sciences Group, Scotland's Rural College,
18 Edinburgh, EH25 9RG, UK; Telephone: +44 (0)131 651 9366; Fax: +44 (0)131
19 535 3121; marie.haskell@sruc.ac.uk

20 Running title: Genetic analysis of behaviour in dogs

21 Word count: 5629

22 **Abstract**

23 A favourable genetic structure and diversity of behavioural features highlights the
24 potential of dogs for studying the genetic architecture of behaviour traits. However,
25 behaviours are complex traits, which have been shown to be influenced by
26 numerous genetic and non-genetic factors, complicating their analysis. In this
27 study, the genetic contribution to behaviour variation in German Shepherd dogs
28 (GSDs) was analysed using genomic approaches. GSDs were phenotyped for
29 behaviour traits using the established Canine Behavioral Assessment and Research
30 Questionnaire (C-BARQ). Genome-wide association study (GWAS) and regional
31 heritability mapping (RHM) approaches were employed to identify associations
32 between behaviour traits and genetic variants, while accounting for relevant non-
33 genetic factors. By combining these complementary methods we endeavoured to
34 increase the power to detect loci with small effects. Several behavioural traits
35 exhibited moderate heritabilities, with the highest identified for Human-directed
36 playfulness, a trait characterised by positive interactions with humans. We
37 identified several genomic regions associated with one or more of the analysed
38 behaviour traits. Some candidate genes located in these regions were previously
39 linked to behavioural disorders in humans, suggesting a new context for their
40 influence on behaviour characteristics. Overall, the results support dogs as a
41 valuable resource to dissect the genetic architecture of behaviour traits and also
42 highlight the value of focusing on a single breed in order to control for background
43 genetic effects and thus avoid limitations of between-breed analyses.

44 Keywords: GWAS, regional heritability mapping, C-BARQ

45 **Introduction**

46 The dog (*Canis familiaris*) is a useful animal model for identifying the genetic
47 basis of various phenotypes (Boyko, 2011; Schoenebeck and Ostrander, 2014) due
48 to its favourable genetic structure, characterised by a high linkage disequilibrium
49 and shared haplotypes across breeds (Karlsson et al., 2007; reviewed in Hall and
50 Wynne, 2012). Behavioural traits of dogs have also been shown to have a genetic
51 component, supported by significant within-breed genetic variance (Ilska et al.,
52 2017), pronounced differences in behavioural characteristics between dog breeds
53 (Mehrkam and Wynne, 2014; Eken Asp et al., 2015) and Belyaev's famous
54 "Farmed Fox" experiment in which silver foxes (close relatives of dogs) were
55 successfully selected over several generations for increased and decreased
56 tameness (Kukekova et al., 2012). Thus, the dog may also be a useful model for
57 characterising the genetic architecture of behaviour and has already been used to
58 gain insights into the genetic mechanisms underlying conditions that are also
59 relevant in humans, such as obsessive-compulsive disorder (Dodman et al., 2010;
60 Tang et al., 2014). In addition to such disorders, dogs may provide unique insights
61 into the genetic basis of complex and general behaviour characteristics, including
62 personality traits (Hall and Wynne, 2012).

63 There are also practical concerns for studying the genetic contribution to behaviour
64 variation in dogs. As the first domesticated species, dogs are still employed in
65 many roles such as herding, hunting, military and police work and serving as guide
66 dogs, but foremost, the special social bond that developed between humans and
67 dogs has led to the dog's popularity as a companion animal. Although dogs show
68 tameness and strong attachment to humans in contrast to their wild ancestors,

69 unwanted behaviours (e.g. excessive aggression, separation anxiety) still occur that
70 affect the welfare of dogs, owners and the public (Rooney and Bradshaw, 2014;
71 Casey et al., 2014; Roth et al., 2016). Numerous studies have been performed with
72 the aim of identifying non-genetic risk factors for the occurrence of unwanted
73 behaviours, such as living conditions and demographic factors (Haverbeke et al.,
74 2008; Blackwell et al., 2008; Rooney and Cowan, 2011; McGreevy et al., 2013;
75 Deldalle and Gaunet, 2014; Tiira and Lohi, 2015; Serpell and Duffy, 2016) but few
76 studies have considered the role of genetic factors in the management of problem
77 behaviours. A better understanding of the genetic basis of dog behaviour may also
78 inform breeding programs for working dogs, e.g. guide dogs (Goddard and
79 Beilharz, 1982).

80 This study aims to gain general insights into the genetic architecture of behaviour
81 variation using German Shepherd dogs (GSDs). The GSDs in this study represent
82 unique samples of pet dogs from the United Kingdom (UK) and from a breeding
83 program of the Swedish Armed Forces (SAF) specifically selected for behaviour
84 traits. By focusing on a single breed and controlling for background genetic
85 structure that might be a consequence of analysing two populations, while also
86 accounting for relevant environmental factors, the limitations of between-breed
87 analyses and confounding with non-genetic effects were minimized. Moreover,
88 different genetic approaches were applied to explore the complex nature of
89 behaviour traits. In addition to employing a genome-wide association study
90 (GWAS) approach based on single SNPs, a regional heritability mapping (RHM)
91 approach was also conducted, which has been shown to perform better in the
92 identification of multiple quantitative trait loci (QTL) with small effects (Nagamine

93 et al., 2012). Our results highlight the complex and polygenic nature of behaviour
94 traits and we also demonstrate that the dog is a valuable resource to study the
95 genetic architecture of behaviour.

96 **Material and Methods**

97 **Samples and phenotypes**

98 Data on GSD behaviour and management was assessed using the Canine
99 Behaviour and Research Questionnaire (C-BARQ) (Hsu and Serpell, 2003) and a
100 lifestyle survey (Friedrich et al., 2018). The C-BARQ consists of 101 questions
101 related to training and obedience, aggression, fear and anxiety, separation-related
102 behaviour, excitability, attachment and attention seeking, and miscellaneous
103 behaviours. The original C-BARQ was extended by 15 questions that assess the
104 dog's playfulness (Svartberg, 2005; Arvelius, Asp, et al., 2014) and 21 of the
105 miscellaneous C-BARQ questions were removed due to a lack of variability
106 (Arvelius, Asp, et al., 2014), leading to 95 final questions.

107 The lifestyle survey consists of questions concerning demographic factors of the
108 dog (e.g., sex, neuter status, age), its living situation (number of children, adults
109 and other animals living with the dog, where the dog is housed) and its current and
110 past management (puppy socialisation, exercise and stimulation, training,
111 activities).

112 Owners of registered UK GSDs that were at least two years old were invited to
113 participate in the study via email by the UK Kennel Club (KC). Participating GSDs
114 from the UK cohort were primarily pet dogs. All GSDs from the Swedish cohort
115 were bred within the breeding program of the SAF. After a behaviour test at the

116 age of 15-18 months, dogs started training for working with the SAF, Swedish
117 Police or other authorities or companies, and/or were selected as breeding animals,
118 whereas others were kept as companions (Wilsson and Sinn, 2012). For the
119 Swedish cohort, owners, trainers or handlers of GSDs bred within the breeding
120 program of the SAF that were at least two years old were invited via email or letter
121 to participate in the study.

122 Behaviour data and demographic and management factors were available for 1,041
123 GSDs from the UK and Sweden (UK=426, Sweden=615). To calculate the
124 behaviour traits, a principal component analysis (PCA) was applied to the data to
125 condense the 95 questions to a smaller number of components (described in
126 Friedrich et al., 2018). Briefly, several procedures (Cattell's scree-test, Horn's
127 Parallel test and the Very Simple Structure (VSS) criterion) were applied and
128 implemented using the R package 'psych' to identify the optimal number of
129 components that capture the important information (Abdi and Williams, 2010),
130 which gave a value of 15 for all tests. The PCA was then run for 15 principal
131 components, followed by a varimax (orthogonal) rotation (for more information
132 see Abdi and Williams, 2010). Missing values in the data set were replaced by the
133 median value. The dogs' scores for the 15 components were considered as
134 quantitative behaviour traits in the subsequent analyses.

135 These 15 traits describe fearful, aggressive and playful behaviours in response to
136 humans or dogs, separation anxiety, attachment and excitability, chasing, touch-
137 sensitivity and obedience (Friedrich et al., 2018). After correcting for fixed effects
138 (see below), the distribution of residuals for two behavioural traits, Aversion of
139 being stepped over and Resource guarding, were significantly skewed due to dogs

140 with extreme values. A Shapiro-Wilk test of normality revealed the highest
141 deviations from a normal distribution for the residuals of these traits and therefore
142 these traits were not considered for the following analyses, leaving 13 traits for
143 further analysis. An overview of the 13 behaviour traits (principal components)
144 used in the subsequent analyses is given in the supplement (S1 Table).

145 **Determination of non-genetic effects**

146 Demographic and management factors were assessed with the lifestyle survey as
147 described previously (Friedrich et al., 2018). Briefly, 28 factors were fitted in an
148 initial linear model for each behaviour trait. Backward elimination was then
149 applied to identify the model with the lowest Akaike information criterion (final
150 model). These behaviour-specific final models were used in the subsequent
151 analyses (S2 Table).

152 **Genotyping and quality control**

153 DNA was extracted for 768 dogs from saliva samples collected with Performagene
154 PG-100 swabs (UK cohort) or blood samples (Swedish cohort) using standard
155 protocols. The genotyping was performed using the Illumina CanineHD Whole-
156 Genome Genotyping BeadChip featuring 172 115 SNPs. When a filter for a sample
157 call rate of > 90% was applied, 745 dogs passed the genotyping quality control.
158 The data set was then checked using sex and relationship information estimated
159 from the genotype data to identify potential sampling errors and 4 further samples
160 were removed. The final data set included 741 dogs (UK=324, Sweden=417) with
161 sex ratios of 0.8 and 0.7 (# males: # females) for UK and Swedish dogs,
162 respectively. SNPs were filtered in GenomeStudio software (Illumina Inc., San

163 Diego) for call rate > 98%, reproducibility (GTS) > 0.6 and signal intensity,
164 characterised by AB R mean (mean normalized intensity of the AB cluster) > 0.3.
165 Using PLINK version 1.9 (Purcell and Chang; Chang et al., 2015), SNPs were also
166 filtered for minor allele frequency (MAF) > 0.05 and lack of evidence for
167 deviations from Hardy-Weinberg equilibrium (Bonferroni-corrected p-value of
168 $0.05 = 4.5 \times 10^{-7}$). Due to allelic imbalance that can cause bias in association
169 studies (discussed in Wise et al., 2013), SNPs on the X chromosome were
170 removed. The final set included 78 088 autosomal SNPs.

171 **Pedigree and population structure**

172 Although the GSDs in this study were from two different countries, there were
173 shared pedigree links. Thus, the UK and Swedish pedigrees were merged into a
174 joint pedigree including both cohorts. To identify underlying population structure
175 in the genomic data, a PCA was performed. To account for linkage disequilibrium
176 between SNPs, a pruned SNP data set was used as input for the PCA, as
177 recommended by PLINK version 1.9 (Purcell and Chang; Chang et al., 2015).
178 Genotype pruning on the filtered data set (78 088 SNPs) was performed using
179 PLINK version 1.9 (Purcell and Chang; Chang et al., 2015) based on the variance
180 inflation factor, a function of the multiple correlation coefficient of a given SNP
181 regressed on all other SNPs within a window (using default parameters: window
182 size = 50 SNPs, the number of SNPs to shift the window at each step = 5, the
183 variance inflation factor threshold = 2), leaving 9 180 SNPs as input for the PCA.
184 The PCA was subsequently carried out in PLINK version 1.9 (Purcell and Chang;
185 Chang et al., 2015).

Estimation of heritability

The heritability (h^2) was estimated using pedigree and genotype data (the filtered data set of 78 088 SNPs). For the pedigree-based estimates, all GSDs with behaviour records ($n = 1\,041$) were used and the joint pedigree for the phenotyped dogs comprised 24 284 dogs. Heritability was estimated in ASReml (Gilmour et al., 2009) and GCTA (Yang et al., 2011) for pedigree- and genotype-based approaches, respectively, by fitting the following model:

$$y = 1\mu + Xb + Za + \varepsilon \quad (1)$$

where y is a vector of behaviour traits, μ is the overall mean, b is a vector of fixed effects with X as the corresponding incidence matrix, Z is the incidence matrix for the random additive polygenic effect, a is a vector of random additive polygenic effects distributed as $MVN(0, \sigma_a^2 A)$ and $MVN(0, \sigma_a^2 G)$ for the pedigree- and genotype-based estimates, respectively, where A is the pedigree-based relationship matrix and G is the genomic relationship matrix. ε is a vector of residual errors distributed as $MVN(0, \sigma_e^2 I)$, where I is an identity matrix. The fixed effects include the demographic and management factors that were detected to best predict the behaviour trait (S2 Table). Dogs for which one or more fixed effects were missing were removed from the analysis, such that the number of GSDs included in the analysis varied across behaviour traits (range of 906 to 1 038 and 638 to 729 for pedigree-based and genotype-based estimations, respectively) (Table 1).

The significance of pedigree-based h^2 was tested using a log-likelihood ratio test (LRT) in ASReml (Gilmour et al., 2009), comparing the log-likelihood ratio statistic to a χ^2 (d.f.=1) for $p < 0.05$. The significance of genotype-based estimates

209 was defined by p-values < 0.05 from the LRT within the genome-based restricted
210 maximum likelihood (GREML) analysis performed in GCTA (Yang et al., 2011).

211 **Genome-wide association study (GWAS)**

212 A GWAS was performed on the filtered data set of 78 088 SNPs to identify
213 associations between SNPs and behaviour traits based on an additive model. To
214 account for population structure, models with different combinations of factors
215 (cohort as fixed effect, genotype-derived principal components 1 and 2 as
216 covariates, genomic relationship matrix as random effect) were evaluated. Fitting
217 only the cohort and the relationship matrix performed best, as assessed by the
218 genomic inflation factor (λ) (i.e. closest to 1.0). The following linear model was
219 fitted in GEMMA (Zhou and Stephens, 2012):

$$220 \quad y = 1\mu + Xb + c\beta + Za + \varepsilon \quad (2)$$

221 where y is a vector of behaviour traits, μ is the overall mean, b is a vector of fixed
222 effects with X as the corresponding incidence matrix, c is a vector of marker
223 genotypes (alleles coded as 0/1) with β as the vector of regression coefficients of
224 the phenotype on the marker genotypes, Z is the incidence matrix for the random
225 additive polygenic effect, a is a vector of random additive polygenic effects with
226 $MVN(0, \sigma_a^2 G)$, where G is the genomic relationship matrix, and ε is a vector of
227 residual errors with $MVN(0, \sigma_e^2 I)$, where I is an identity matrix. The fixed effects
228 comprise the demographic and management factors obtained in the individual final
229 models (S2 Table).

230 A conservative Bonferroni correction was applied to determine genome-wide
231 significance ($P < \frac{0.05}{78\,088}$; 6.4E-07) and suggestive (allowing one false positive per
232 genome scan: $P < \frac{1}{78\,088}$; 1.3E-05) (Riggio et al., 2013) thresholds that account for
233 the multiple testing resulting from the large number of markers but not for multiple
234 behaviour traits.

235 **Regional heritability mapping (RHM)**

236 Genomic regions were also tested for association with behaviour traits. This was
237 carried out by scanning windows across the whole genome using RHM, performed
238 in REACTA (Gray et al., 2012). This approach used the model described by
239 Nagamine et al. (2012) where two genetic effects are fitted: the first representing
240 the overall genetic effects (modelled with an overall genomic relationship matrix
241 calculated using all SNPs across the genome) and the second genetic effect
242 representing the effect associated with the specific region of the genome being
243 tested (modelled with a regional genomic relationship matrix calculated using only
244 SNPs from this region). The SNPs used for the regional relationship matrix were
245 excluded from the overall genomic relationship matrix (Cebamanos et al., 2014).
246 REACTA (Gray et al., 2012) uses a sliding window approach and we used a fixed
247 window size of 50 SNPs with overlaps of 25 SNPs. The window size of 50 SNPs
248 was chosen as a compromise between power to detect associations and
249 computational demands (Uemoto et al., 2013).

250 Using these parameters resulted in 3 124 regions under analysis; to correct for
251 multiple testing, a Bonferroni correction was applied to genome-wide significance
252 ($P < \frac{0.05}{3\ 124}$; 1.6E-05) and suggestive ($P < \frac{1}{3\ 124}$; 3.1E-04) thresholds.

253 **Analysis of candidate genes and regions**

254 The coordinates of identified SNPs and regions were mapped to the CanFam3.1
255 assembly to identify (I) genes harbouring or near identified SNPs (GWAS) and (II)
256 genes located within identified regions (RHM). Regarding (I): to determine the size
257 of the region around identified SNPs that should be scanned for candidate genes,
258 the squared correlation (r^2) between all pairs of SNPs within 10Mb were calculated
259 across the genome using PLINK version 1.9 (Purcell and Chang; Chang et al.,
260 2015). The average r^2 was calculated for bins of increasing distance between SNPs
261 to identify the distance around SNPs at which average r^2 drops below 0.5. The
262 longest bin for which average $r^2 > 0.5$ was 200 kb and thus this distance was chosen
263 as the region around associated SNPs to be investigated. Regarding (II), the GWAS
264 results, $-\log_{10}(P)$, were plotted within the regions identified by RHM to identify
265 positional candidate genes. The pairwise r^2 was calculated between all SNPs in the
266 region and the SNP with highest $-\log(P)$ value to describe the pattern of linkage for
267 the region, using PLINK version 1.9 (Purcell and Chang; Chang et al., 2015) as
268 described above. The regional associations plots were created using an R script
269 modified from that of Saxena et al. (2007).

270 All genes within the regions described above (I and II) were submitted to Enrichr
271 (Chen et al., 2013; Kuleshov et al., 2016) to identify enriched biological processes.

272 **Results**

273 **Population structure**

274 We explored the underlying population structure in the two GSD cohorts by
275 applying a PCA to the genomic data. The variance in the genomic data explained
276 by the first three principal components was 2.18%, 1.68% and 1.22%, respectively,
277 and 66.96% of the variance was explained by all components with eigenvalue > 1.
278 Plotting the first two components of the PCA (S3 Figure) shows population
279 structure by cohort by a clear separation of UK and Swedish dogs based on the first
280 principal component. However, some GSDs overlapped between the cohorts,
281 showing shared ancestry. In contrast to the cohort effect, there were no distinct
282 patterns observable for eigenvectors PC1 and PC2 when considering the GSDs
283 according to their function or coat colour.

284 **Heritabilities**

285 Heritability estimates for the 13 behaviour traits were calculated using pedigree
286 and genomic data. Moderate and significant h^2 were found for Human-directed
287 playfulness and Non-social fear using pedigree and genomic approaches, while
288 Stranger-directed interest was only significant for pedigree-based estimates and
289 Chasing only for genomic estimates (Table 1). The highest h^2 were calculated for
290 Human-directed playfulness using pedigree data (0.23 ± 0.08) and for Non-social
291 fear using genotype data (0.16 ± 0.06). Non-significant heritabilities were
292 estimated for Stranger-directed fear, Excitability, Attachment/ Attention seeking,
293 Dog-directed fear and Touch-sensitivity using estimates from pedigree and
294 genomic data.

295 **Association mapping**

296 Genome-wide association studies (GWAS) and a regional heritability mapping
297 (RHM) were performed as complementary approaches to identify associations
298 between genetic markers and the 13 behaviour traits (Figure 1). The average
299 genomic inflation for GWAS across the 13 behaviour traits was 0.99 (ranging from
300 0.89 to 1.06), showing that population stratification was adequately controlled (S4
301 Figure). In the GWAS, a total of 15 SNPs were found with a suggestive association
302 to one of the analysed behaviour traits and two of these also showed a genome-
303 wide significant association ($P < 6.4E-07$) (Table 2).

304 The identified SNPs were distributed over 7 of the 38 canine autosomes, with the
305 largest numbers on CFA33 (5) for Attachment/Attention seeking, 31 (3) for Dog-
306 directed fear and 14 (3) for Stranger-directed interest. The genome-wide
307 associations were found for Attachment/Attention seeking (2 adjacent SNPs on
308 CFA33). The greatest number of suggestive SNPs were found for Attachment/
309 Attention seeking (6), Stranger-directed interest (3) and Dog-directed fear (3).

310 The RHM analysis was performed by testing for associations between 50-SNP
311 sliding windows across the genome (with a 25-SNP overlap between consecutive
312 windows) (Figure 1). Scanning the genome for regions associated with the 13
313 behaviour traits based on the suggestive threshold, we identified 16 regions
314 associated with at least one of the behaviour traits (Table 3). One region on CFA33
315 associated with Attachment/Attention seeking showed genome-wide significance
316 and also harbours the only SNPs with genome-wide significance in the GWAS.
317 The average size of the identified regions was 1.31 Mb (range: 0.89-2.63 Mb).

318 Most of the SNPs identified by the GWAS overlapped with regions identified by
319 the RHM (Table 2; Table 3; Figure 1), only the SNPs found on CFA10 and CFA17
320 for Dog-directed aggression and on CFA31 for Dog-directed fear were exclusive to
321 the GWAS approach. Exclusive peaks were also found with the RHM approach,
322 for example on CFA1 for Separation-anxiety, on CFA3 for Chasing, and on CFA19
323 for Excitability.

324 **Candidate genes and regions**

325 According to the annotation of CanFam3.1, four of the SNPs identified by the
326 GWAS were located within three genes (*ARNT*, *PLCH1* and *BRWD1*) and 30 genes
327 were located within 200 kb of suggestive or genome-wide significant SNPs (Table
328 2). The two SNPs on CFA33 with genome-wide significance for
329 Attachment/Attention seeking are located approximately 63 kb downstream of an
330 unannotated protein-coding gene (*ENSCAFG00000009706*). Gene ontology analysis
331 of the 30 genes revealed that the top enriched biological processes are
332 “polyphosphate metabolic process” (GO: 0006797; adjusted p-value = 0.009),
333 “negative regulation of axon regeneration” (GO: 0048681; adjusted p-value = 0.12)
334 and “regulation of hormone biosynthetic process” (GO: 0046885; adjusted p-value
335 = 0.12).

336 To further investigate regions identified by the RHM analysis, $-\log(P)$ values
337 obtained from the GWAS, gene annotations and local linkage disequilibrium
338 patterns were plotted for these regions to pinpoint the most likely location of
339 positional candidate genes (S5 Figure). Overlapping regions, due to the sliding
340 window approach of the RHM analysis, were combined. There were 60 genes

341 located in these regions (Table 3); of these, several functional candidate genes
342 (*LRRN3*, *KCNAB1* and *BRWD1*) were also located near (S5 Figure) or at (Table 2)
343 SNPs identified by GWAS. Two other functional candidate genes (*HIVEP2* and
344 *AIG1*) were located in identified regions but the $-\log(P)$ values for nearby SNPs
345 obtained in the GWAS did not exceed the suggestive threshold (S5 Figure). The
346 region on CFA33 with genome-wide significance for Attachment/Attention seeking
347 comprised three unannotated protein-coding genes (*ENSCAFG00000009682*,
348 *ENSCAFG00000009697* and *ENSCAFG00000009706*).

349 According to the gene ontology analysis, the GO biological processes significantly
350 enriched by genes located in identified regions (Table 3) are “histidine catabolic
351 process” (GO: 0006548; adjusted p-value = 0.013), “histidine metabolic process”
352 (GO: 0006547; adjusted p-value = 0.013) and “imidazole-containing compound
353 catabolic process” (GO: 0052805; adjusted p-value = 0.013).

354 **Discussion**

355 Dogs express diverse behaviour phenotypes, some of which appear to be related to
356 traits of other species (including humans), making them useful models for general
357 insights into the genetic architecture of behaviour. However, behaviours are
358 complex traits, which have been shown to be influenced by numerous non-genetic
359 (environmental) factors and genetic variants of low to moderate effect (Flint,
360 2003), which complicates their analysis and the identification of underlying genes
361 and mechanisms. In this study, we analysed the influence of genetic factors on
362 behaviour traits of German Shepherd dogs using multiple genomic approaches,

363 while accounting for various non-genetic factors, with the aims of characterising
364 the general genetic architecture of behaviour and identifying candidate genes.

365 **The genetic contribution to behaviour variation**

366 The heritabilities estimated for the 13 behaviour traits using pedigree and genomic
367 approaches ranged from 0 to 0.23. These measures for h^2 are within the range of
368 most previously observed values in dogs (Saetre et al., 2006; Arvelius, Strandberg,
369 et al., 2014; Ilkka et al., 2017), while a few studies reported higher h^2 for similar
370 behaviour traits (Ruefenacht et al., 2002; van der Waaij et al., 2008). Discrepancies
371 between observed h^2 for dog behaviour traits across studies can be explained by the
372 different behaviour phenotypes used, e.g. whether the behaviour was subjectively
373 scored or actually measured and whether the behaviour was recorded in everyday
374 life or in test situations, and also by differences between breeds (due to different
375 population histories).

376 From other species it is known that specific behaviour patterns contributing to the
377 fitness of an individual, such as courtship or feeding, are under stronger genetic
378 control than behaviours with apparently less evolutionary relevance like
379 personality traits (York, 2018). In this study, behaviour traits with substantial h^2
380 were Human-directed playfulness, Non-social fear, Stranger-directed interest and
381 Chasing. The observation of the highest h^2 across traits for Human-directed
382 playfulness has been also made in a genetic study of 14 different dog breeds (Asp
383 et al., 2014). While many other studies on the genetic background of dog behaviour
384 focused on human-directed aggression (Liinamo et al., 2007; Våge et al., 2010;
385 Zapata et al., 2016), we included traits of playful interactions in our analysis since
386 playfulness in regard to humans has been shown to explain a large proportion of

387 the variance between individuals in the analysis of multiple dog breeds (Svartberg,
 388 2005). In particular, Human-directed playfulness and Stranger-directed interest
 389 describe boldness and attachment to humans and our results indicate that these
 390 behaviour characteristics might be directly targeted by selection for tameness and
 391 human-attachment in dogs. Specifically regarding GSDs, although the SAF do not
 392 use C-BARQ for their selection programme, a previous study showed significant
 393 associations between success in a temperament test assessing dogs for further
 394 training and C-BARQ-measured traits of young dogs related to Lack of obedience,
 395 Stranger-directed fear, Non-social fear, Dog-directed fear and Touch sensitivity
 396 (Foyer et al., 2014), suggesting that these traits have been selected against in the
 397 Swedish cohort. We do not have similar information for the UK cohort as these
 398 dogs are primarily pets and not part of a breeding programme, however, it is
 399 possible that selection criteria over recent years have been based more on cosmetic
 400 traits as the breed has moved from a working dog to pet (O'Neill et al., 2017).

401 Using genome-wide association and regional heritability mapping, we identified 15
 402 SNPs and 16 regions, respectively, which showed suggestive association with one
 403 of the analysed behaviour traits. These SNPs and regions were distributed over 11
 404 chromosomes. Several regions were identified by both GWAS and RHM.

405 Comparing genomic regions identified in the current study to the results from other
 406 single-breed studies, we found that the SNP for Attachment/Attention seeking on
 407 CFA7 is located in a region of approximately 1 Mb flanked by two loci associated
 408 with obsessive-compulsive disorder in Doberman Pinschers (Tang et al., 2014). In
 409 contrast, the suggestive SNPs identified for behaviour traits in Labrador Retrievers
 410 by Iliska et al. (2017) do not overlap with candidate regions found in the current

411 study. Furthermore, none of the genetic regions mapped to aggression and fear
412 across multiple dog breeds in a study by Zapata et al. (2016) overlapped with
413 genetic regions found in the current study. Ostrander et al. (2017) reviewed the
414 identified loci for behaviour traits across dog breeds by Zapata et al. (2016) and
415 found that many of these loci were previously linked to body size, suggesting that
416 behaviour may have been confounded with physical characteristics in between-
417 breed analyses or an association between behaviour and some morphological traits.
418 In the silver fox experiment described above, changes in behaviour were also
419 accompanied by physiological and morphological changes (Trut, 1999) and other
420 studies have shown an association between behaviour and body traits across breeds
421 (McGreevy et al., 2013), suggesting an genetic interplay between these traits.
422 These observations might also indicate that GWAS across dog breeds are more
423 sensitive for morphological differences than for variation in behaviour, which
424 highlights the importance of single-breed analyses in the dissection of the genetic
425 background of behaviour. In contrast to the Zapata et al. (2016) study, candidate
426 regions identified in the current study do not overlap with known genetic regions
427 associated with body size (based on the largest study to date, Hayward et al., 2016).

428 However, our results also suggest that QTL for dog behaviour may be breed-
429 specific as indicated by the lack of QTL that overlap those found in other studies. It
430 is likely that across breeds, different behaviour-oriented breeding practices have
431 led to different alleles selected to moderate frequencies, leading to breed-specific
432 QTL.

433 **Candidate genes related to behaviour traits**

434 In this study, we combined two complementary approaches (GWAS and RHM)
435 with the aim of detecting novel candidate genes for behaviour and further
436 evaluating genes previously linked to behaviour.

437 The only SNPs and region with genome-wide significance for the behaviour trait
438 Attachment/ Attention seeking point to a region on CFA33 that contains several
439 unannotated protein-coding genes, including *ENSCAFG00000009706*. According
440 to the iDOG database (Tang et al., 2019), *ENSCAFG00000009706* is a protein-
441 coding gene with molecular functions related to RNA binding and the structural
442 constitution of the ribosome (GO: 0003723 and 0003735). However, this gene has
443 not yet been described in other canine association mapping studies.

444 Many of the other positional candidate genes have been previously linked to
445 behaviour characteristics and disorders or to neuronal development, especially in
446 regards to humans. The aquaporin-4 (*AQP4*) gene identified by both GWAS and
447 RHM for Attachment/Attention-seeking is one of the most abundant molecules in
448 the brain, with many physiological functions (reviewed in Nagelhus and Ottersen,
449 2013). In a study on gene expression changes in the brains of dogs and wolves,
450 *AQP4* showed a significant 4-fold higher gene expression in dog than in wolf,
451 indicating that it may have played a role in domestication (Saetre et al., 2004). Our
452 results provide further evidence for the role of this gene regarding attachment to
453 humans.

454 RHM identified several regions that were not identified by the GWAS and contain
455 genes that have previously been linked to behaviour. The region at ~34 Mb on

456 CFA1, associated with Separation anxiety, includes *HIVEP2* and *AIG2*, which have
457 been previously identified as positional candidate genes in a GWAS on affiliative
458 social behavior in humans (Knoll et al., 2018). The region at 50-52 Mb on CFA14,
459 associated with Stranger-directed interest, includes *LRRN3*, a strong risk gene for
460 autism in humans (Hutcheson et al., 2004). In addition, the region at ~49-51 Mb on
461 CFA23, associated with Touch-sensitivity (a behaviour trait that is characterised by
462 fearful or aggressive responses to grooming or bathing), contains another
463 promising functional candidate gene, *KCNAB1*. Two SNPs with low but not quite
464 suggestive p-values in the GWAS were also located within the *KCNAB1* gene,
465 which encodes the voltage-gated potassium channel subunit beta-1. Interestingly,
466 mouse knockouts at the *KCNQ* gene, which encodes another voltage-gated
467 potassium channel, showed an increased sensitivity of mechanoreceptors in the
468 skin (Schütze et al., 2016). It is possible that variation in *KCNAB1* could have a
469 similar effect and thus this might influence touch-sensitivity in dogs.

470 The GO analysis for genes identified by the RHM revealed an enrichment of
471 catabolic and metabolic histidine processes due to the genes *AMDHD1* and *HAL*
472 (the region harbouring these two genes was associated with Stranger-directed fear).
473 Histidine is a precursor of the neurotransmitter histamine and it has been shown
474 that the histaminergic system affects the central nervous system and thus also alters
475 behaviours, e.g. by affecting the fear-memory (reviewed in Passani et al., 2007).

476 Other genes were identified only by the GWAS, including *BRWD1* (CFA31),
477 *B3GALT5* (CFA31) and *ARNT* (CFA17). Two SNPs associated with Dog-directed
478 fear are located within *BRWD1*. In human GWAS studies, this gene has been
479 associated with cognitive function (Davies et al., 2018), intelligence (Savage et al.,

2018) and temperament in individuals with a bipolar disorder (Greenwood et al., 2012). In close proximity to these SNPs lies *B3GALT5*, which has been linked to suicide attempts (Perlis et al., 2010) and obsessive-compulsive symptoms (den Braber et al., 2016). Finally, a SNP on CFA17 associated with Stranger-directed interest is located within the *ARNT* gene. Variation within *ARNT* has been linked to the severity of autism in humans (Fujisawa et al., 2016).

Limitations and implications for further studies

The limited number of genome-wide significant associations found in this study indicates the challenges in the genetic dissection of complex traits like behaviour, which derive from the small effects of genetic variants on phenotypic variation, substantial environmental effects and difficulties in defining clear phenotypes. Although ours is one of the largest genomic studies of dog behaviour so far, it has been shown in human studies that much larger sample sizes are required for robust genetic dissection of complex traits, e.g. height (Visscher et al., 2014). The use of C-BARQ, a standardised owner-derived questionnaire, to measure behaviour phenotypes, which has been successfully applied in many studies and records a range of behaviours in everyday situations, opens the possibility of meta-analysis across studies and thus ultimately achieving a larger sample size. However, a limitation of using questionnaire-based phenotypes is that the recorded traits are influenced by the subjectivity of the participants, which might be even more pronounced when participants originate from different countries and thus show cultural differences as in this study. While we attempted to correct for this in the statistical analysis, we may not have been completely successful.

503 **Conclusions**

504 Understanding the genetics of dog behaviour and the interaction with non-genetic
505 factors can give general insights into animal and human behaviour and is relevant
506 for animal welfare, e.g. to identify risk factors for problem behaviours. Our results
507 support the hypothesis that behaviours are complex traits, influenced by multiple
508 genetic and non-genetic factors, emphasizing the need for large datasets
509 incorporating both genetic and non-genetic information in future studies of dog
510 behaviour. Furthermore, it is important to reach a consensus on the non-genetic
511 factors with greatest effects on these traits in order to standardise analyses.

512 If these requirements are met, dogs can provide a valuable resource for studying
513 the genetics of behaviour characteristics, especially in terms of intra- and inter-
514 species social interactions. In this study, genomic regions and SNPs associated
515 with behaviour traits suggested a number of candidate genes that were previously
516 described for psychological disorders in humans, indicating a potential new context
517 for these genes in the general expression of behaviour variation. By analysing a
518 single dog breed, we were able to highlight candidate genes for behaviour that are
519 less likely to be confounded with morphological variation compared to between-
520 breed analyses. However, further studies with larger sample sizes are required to
521 identify and confirm the identified associations and candidate genes and, where
522 associations are confirmed, subsequent functional analyses will be needed to
523 progress in understanding how these genes influence expression of behaviour.

524

525 Supplementary information is available at Heredity's website.

526 **Acknowledgements**

527 The authors want to thank all owners of German Shepherd dogs participating in
528 this study for their time and effort to answer the questionnaires and send saliva
529 samples for genotyping. Thanks are also extended to the Kennel Club, the British
530 Association for German Shepherd Dogs, and the German Shepherd Dog Breed
531 Council of Great Britain for assistance in participant recruitment for the UK cohort.
532 Thanks to Zita Polgar, Carol-Anne Duthie and Joanna Warner for assistance in
533 contacting dog owners. We would also like to thank the SAF Dog Training Centre,
534 in particular Lisa Rutström, for recruiting participants for the Swedish cohort, and
535 Susanne Gustafsson and Gabriela Bottani Claros (Swedish University of
536 Agricultural Sciences) for providing DNA samples. We thank Helen Brown for
537 statistical advice and Dr. James Serpell (University of Pennsylvania, USA) for
538 permission to use C-BARQ. Primary funding was provided by the Dogs Trust
539 (UK); further funding was provided by BBSRC Institute Strategic Programme
540 Grants (to the Roslin Institute) and RESAS, Scottish Government (to SRUC).

541

542 **Conflict of Interest**

543 The authors declare no conflicts of interest.

544

545 **Data archiving**

546 The genotype and phenotype data used in this study will be accessible via Dryad
547 once the paper is accepted.

548

549 **References**

- 550 Abdi H, Williams LJ (2010). Principal component analysis. *Wiley Interdiscip Rev*
551 *Comput Stat* **2**: 433–459.
- 552 Arvelius P, Asp HE, Fikse WF, Strandberg E, Nilsson K (2014). Genetic analysis
553 of a temperament test as a tool to select against everyday life fearfulness in Rough
554 Collie. *J Anim Sci* **92**: 4843–4855.
- 555 Arvelius P, Strandberg E, Fikse WF (2014). The Swedish Armed Forces
556 temperament test gives information on genetic differences among dogs. *J Vet*
557 *Behav* **9**: 281–289.
- 558 Asp HE, Arvelius P, Fikse WF, Nilsson K, Strandberg E (2014). Genetics of
559 Aggression, Fear and Sociability in Everyday Life of Swedish Dogs. *Proc World*
560 *Congr Genet Appl Livest Prod Species Breeding: Companion Animals (Posters)*:
561 795.
- 562 Blackwell EJ, Twells C, Seawright A, Casey RA (2008). The relationship between
563 training methods and the occurrence of behavior problems, as reported by owners,
564 in a population of domestic dogs. *J Vet Behav Clin Appl Res* **3**: 207–217.
- 565 Boyko AR (2011). The domestic dog: man’s best friend in the genomic era.
566 *Genome Biol* **12**: 216.
- 567 den Braber A, Zilhão NR, Fedko IO, Hottenga J-J, Pool R, Smit DJA, et al. (2016).
568 Obsessive–compulsive symptoms in a large population-based twin-family sample

569 are predicted by clinically based polygenic scores and by genome-wide SNPs.
570 *Transl Psychiatry* **6**: e731.

571 Casey RA, Loftus B, Bolster C, Richards GJ, Blackwell EJ (2014). Human directed
572 aggression in domestic dogs (*Canis familiaris*): Occurrence in different contexts
573 and risk factors. *Appl Anim Behav Sci* **152**: 52–63.

574 Cebamanos L, Gray A, Stewart I, Tenesa A (2014). Regional heritability advanced
575 complex trait analysis for GPU and traditional parallel architectures.
576 *Bioinformatics* **30**: 1177–1179.

577 Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ (2015). Second-
578 generation PLINK: rising to the challenge of larger and richer datasets.
579 *GigaScience* **4**: 7.

580 Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, et al. (2013). Enrichr:
581 interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC*
582 *Bioinformatics* **14**: 128.

583 Davies G, Lam M, Harris SE, Trampush JW, Luciano M, Hill WD, et al. (2018).
584 Study of 300,486 individuals identifies 148 independent genetic loci influencing
585 general cognitive function. *Nat Commun* **9**.

586 Deldalle S, Gaunet F (2014). Effects of 2 training methods on stress-related
587 behaviors of the dog (*Canis familiaris*) and on the dog–owner relationship. *J Vet*
588 *Behav Clin Appl Res* **9**: 58–65.

589 Dodman NH, Karlsson EK, Moon-Fanelli A, Galdzicka M, Perloski M, Shuster L,
 590 et al. (2010). A canine chromosome 7 locus confers compulsive disorder
 591 susceptibility. *Mol Psychiatry* **15**: 8–10.

592 Eken Asp H, Fikse WF, Nilsson K, Strandberg E (2015). Breed differences in
 593 everyday behaviour of dogs. *Appl Anim Behav Sci* **169**: 69–77.

594 Flint J (2003). Analysis of quantitative trait loci that influence animal behavior. *J*
 595 *Neurobiol* **54**: 46–77.

596 Foyer P, Bjällerhag N, Wilsson E, Jensen P (2014). Behaviour and experiences of
 597 dogs during the first year of life predict the outcome in a later temperament test.
 598 *Appl Anim Behav Sci* **155**: 93–100.

599 Friedrich J, Arvelius P, Strandberg E, Polgar Z, Wiener P, Haskell MJ (2018). The
 600 interaction between behavioural traits and demographic and management factors in
 601 German Shepherd dogs. *Appl Anim Behav Sci*.

602 Fujisawa TX, Nishitani S, Iwanaga R, Matsuzaki J, Kawasaki C, Tochigi M, et al.
 603 (2016). Association of Aryl Hydrocarbon Receptor-Related Gene Variants with the
 604 Severity of Autism Spectrum Disorders. *Front Psychiatry* **7**.

605 Gilmour AR, Gogel BJ, Cullis BR, Thompson R (2009). *ASReml User Guide*
 606 Release 3.0. VSN International Ltd: Hemel Hempstead, HP1 1ES, UK.

607 Goddard ME, Beilharz RG (1982). Genetic and environmental factors affecting the
 608 suitability of dogs as Guide Dogs for the Blind. *Theor Appl Genet* **62**: 97–102.

609 Gray A, Stewart I, Tenesa A (2012). Advanced Complex Trait Analysis.
610 *Bioinformatics* **28**: 3134–3136.

611 Greenwood TA, Akiskal HS, Akiskal KK, Kelsoe JR (2012). Genome-wide
612 Association Study of Temperament in Bipolar Disorder Reveals Significant
613 Associations To Three Novel Loci. *Biol Psychiatry* **72**: 303–310.

614 Hall NJ, Wynne CDL (2012). The canid genome: behavioral geneticists' best
615 friend? *Genes Brain Behav* **11**: 889–902.

616 Haverbeke A, Laporte B, Depiereux E, Giffroy J-M, Diederich C (2008). Training
617 methods of military dog handlers and their effects on the team's performances.
618 *Appl Anim Behav Sci* **113**: 110–122.

619 Hayward JJ, Castelhana MG, Oliveira KC, Corey E, Balkman C, Baxter TL, et al.
620 (2016). Complex disease and phenotype mapping in the domestic dog. *Nat*
621 *Commun* **7**: 10460.

622 Hsu Y, Serpell JA (2003). Development and validation of a questionnaire for
623 measuring behavior and temperament traits in pet dogs. *J Am Vet Med Assoc* **223**:
624 1293–1300.

625 Huthcheson HB, Olson LM, Bradford Y, Folstein SE, Santangelo SL, Sutcliffe JS, et
626 al. (2004). Examination of NRCAM, LRRN3, KIAA0716, and LAMB1 as autism
627 candidate genes. *BMC Med Genet* **5**: 12.

628 Iliska J, Haskell MJ, Blott SC, Sánchez-Molano E, Polgar Z, Lofgren SE, et al.
 629 (2017). Genetic Characterisation of Dog Personality Traits. *Genetics:*
 630 *genetics.116.192674*.

631 Karlsson EK, Baranowska I, Wade CM, Salmon Hillbertz NHC, Zody MC,
 632 Anderson N, et al. (2007). Efficient mapping of mendelian traits in dogs through
 633 genome-wide association. *Nat Genet* **39**: 1321–1328.

634 Knoll AT, Jiang K, Levitt P (2018). Quantitative trait locus mapping and analysis
 635 of heritable variation in affiliative social behavior and co-occurring traits. *Genes*
 636 *Brain Behav* **17**.

637 Kukekova AV, Temnykh SV, Johnson JL, Trut LN, Acland GM (2012). *Genetics*
 638 of behavior in the silver fox. *Mamm Genome Off J Int Mamm Genome Soc* **23**:
 639 164–177.

640 Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al.
 641 (2016). Enrichr: a comprehensive gene set enrichment analysis web server 2016
 642 update. *Nucleic Acids Res* **44**: W90-97.

643 Liinamo A-E, van den Berg L, Leegwater PAJ, Schilder MBH, van Arendonk
 644 JAM, van Oost BA (2007). Genetic variation in aggression-related traits in Golden
 645 Retriever dogs. *Appl Anim Behav Sci* **104**: 95–106.

646 McGreevy PD, Georgevsky D, Carrasco J, Valenzuela M, Duffy DL, Serpell JA
 647 (2013). Dog Behavior Co-Varies with Height, Bodyweight and Skull Shape. *PLOS*
 648 *ONE* **8**: e80529.

649 Mehrkam LR, Wynne C (2014). Behavioral differences among breeds of domestic
 650 dogs (*Canis lupus familiaris*): Current status of the science. *Appl Anim Behav Sci*
 651 **155**: 12–27.

652 Nagamine Y, Pong-Wong R, Navarro P, Vitart V, Hayward C, Rudan I, et al.
 653 (2012). Localising Loci underlying Complex Trait Variation Using Regional
 654 Genomic Relationship Mapping. *PLOS ONE* **7**: e46501.

655 Nagelhus EA, Ottersen OP (2013). Physiological Roles of Aquaporin-4 in Brain.
 656 *Physiol Rev* **93**: 1543–1562.

657 O'Neill DG, Coulson NR, Church DB, Brodbelt DC (2017). Demography and
 658 disorders of German Shepherd Dogs under primary veterinary care in the UK.
 659 *Canine Genet Epidemiol* **4**: 7.

660 Passani MB, Giannoni P, Bucherelli C, Baldi E, Blandina P (2007). Histamine in
 661 the brain: Beyond sleep and memory. *Biochem Pharmacol* **73**: 1113–1122.

662 Perlis RH, Huang J, Purcell S, Fava M, Rush AJ, Sullivan PF, et al. (2010).
 663 Genome-Wide Association Study of Suicide Attempts in Mood Disorder Patients.
 664 *Am J Psychiatry* **167**: 1499–1507.

665 Purcell SM, Chang CC PLINK 1.9.

666 Riggio V, Matika O, Pong-Wong R, Stear MJ, Bishop SC (2013). Genome-wide
 667 association and regional heritability mapping to identify loci underlying variation
 668 in nematode resistance and body weight in Scottish Blackface lambs. *Heredity* **110**:
 669 420–429.

670 Rooney N, Bradshaw J (2014). Canine Welfare Science: An Antidote to Sentiment
 671 and Myth. In: Horowitz A (ed) Domestic Dog Cognition and Behavior, Springer
 672 Berlin Heidelberg, pp 241–274.

673 Rooney NJ, Cowan S (2011). Training methods and owner–dog interactions: Links
 674 with dog behaviour and learning ability. *Appl Anim Behav Sci* **132**: 169–177.

675 Roth LSV, Faresjö Å, Theodorsson E, Jensen P (2016). Hair cortisol varies with
 676 season and lifestyle and relates to human interactions in German shepherd dogs.
 677 *Sci Rep* **6**.

678 Ruefenacht S, Gebhardt-Henrich S, Miyake T, Gaillard C (2002). A behaviour test
 679 on German Shepherd dogs: heritability of seven different traits. *Appl Anim Behav*
 680 *Sci* **79**: 113–132.

681 Saetre P, Lindberg J, Leonard JA, Olsson K, Pettersson U, Ellegren H, et al.
 682 (2004). From wild wolf to domestic dog: gene expression changes in the brain. *Mol*
 683 *Brain Res* **126**: 198–206.

684 Saetre P, Strandberg E, Sundgren P-E, Pettersson U, Jazin E, Bergström TF (2006).
 685 The genetic contribution to canine personality. *Genes Brain Behav* **5**: 240–248.

686 Savage JE, Jansen PR, Stringer S, Watanabe K, Bryois J, Leeuw CA de, et al.
 687 (2018). Genome-wide association meta-analysis in 269,867 individuals identifies
 688 new genetic and functional links to intelligence. *Nat Genet* **50**: 912.

689 Saxena R, Voight BF, Lyssenko V, Burt NP, Bakker PIW de, Chen H, et al.
690 (2007). Genome-Wide Association Analysis Identifies Loci for Type 2 Diabetes
691 and Triglyceride Levels. *Science* **316**: 1331–1336.

692 Schoenebeck JJ, Ostrander EA (2014). Insights into Morphology and Disease from
693 the Dog Genome Project. *Annu Rev Cell Dev Biol* **30**: 535–560.

694 Schütze S, Orozco IJ, Jentsch TJ (2016). KCNQ Potassium Channels Modulate
695 Sensitivity of Skin Down-hair (D-hair) Mechanoreceptors. *J Biol Chem* **291**: 5566–
696 5575.

697 Serpell JA, Duffy DL (2016). Aspects of Juvenile and Adolescent Environment
698 Predict Aggression and Fear in 12-Month-Old Guide Dogs. *Front Vet Sci* **3**.

699 Svartberg K (2005). A comparison of behaviour in test and in everyday life:
700 evidence of three consistent boldness-related personality traits in dogs. *Appl Anim*
701 *Behav Sci* **91**: 103–128.

702 Tang R, Noh HJ, Wang D, Sigurdsson S, Swofford R, Perloski M, et al. (2014).
703 Candidate genes and functional noncoding variants identified in a canine model of
704 obsessive-compulsive disorder. *Genome Biol* **15**: R25.

705 Tang B, Zhou Q, Dong L, Li W, Zhang X, Lan L, et al. (2019). iDog: an integrated
706 resource for domestic dogs and wild canids. *Nucleic Acids Res* **47**: D793–D800.

707 Tiira K, Lohi H (2015). Early Life Experiences and Exercise Associate with
708 Canine Anxieties. *PloS One* **10**: e0141907.

709 Trut LN (1999). Early Canid Domestication: The Farm-Fox Experiment: Foxes
 710 bred for tamability in a 40-year experiment exhibit remarkable transformations that
 711 suggest an interplay between behavioral genetics and development. *Am Sci* **87**:
 712 160–169.

713 Uemoto Y, Pong-Wong R, Navarro P, Vitart V, Hayward C, Wilson JF, et al.
 714 (2013). The power of regional heritability analysis for rare and common variant
 715 detection: simulations and application to eye biometrical traits. *Front Genet* **4**.

716 Våge J, Wade C, Biagi T, Fatjó J, Amat M, Lindblad-Toh K, et al. (2010).
 717 Association of dopamine- and serotonin-related genes with canine aggression.
 718 *Genes Brain Behav* **9**: 372–378.

719 Visscher PM, Hemani G, Vinkhuyzen AAE, Chen G-B, Lee SH, Wray NR, et al.
 720 (2014). Statistical Power to Detect Genetic (Co)Variance of Complex Traits Using
 721 SNP Data in Unrelated Samples. *PLOS Genet* **10**: e1004269.

722 van der Waaij EH, Wilsson E, Strandberg E (2008). Genetic analysis of results of a
 723 Swedish behavior test on German Shepherd Dogs and Labrador Retrievers. *J Anim*
 724 *Sci* **86**: 2853–2861.

725 Wilsson E, Sinn DL (2012). Are there differences between behavioral
 726 measurement methods? A comparison of the predictive validity of two ratings
 727 methods in a working dog program. *Appl Anim Behav Sci* **141**: 158–172.

728 Wise AL, Gyi L, Manolio TA (2013). eXclusion: Toward Integrating the X
729 Chromosome in Genome-wide Association Analyses. *Am J Hum Genet* **92**: 643–
730 647.

731 Yang J, Lee SH, Goddard ME, Visscher PM (2011). GCTA: a tool for genome-
732 wide complex trait analysis. *Am J Hum Genet* **88**: 76–82.

733 York RA (2018). Assessing the Genetic Landscape of Animal Behavior. *Genetics*
734 **209**: 223–232.

735 Zapata I, Serpell JA, Alvarez CE (2016). Genetic mapping of canine fear and
736 aggression. *BMC Genomics* **17**: 572.

737 Zhou X, Stephens M (2012). Genome-wide Efficient Mixed Model Analysis for
738 Association Studies. *Nat Genet* **44**: 821–824.

739

740 **Figures legends**

741 **Figure 1. Joint Manhattan plots for GWAS and RHM analyses for the 13**
742 **analysed behaviour traits.** Negative log p-values for each SNP and region were
743 plotted according to their chromosomal position for the GWAS (upper plot) and the
744 RHM (lower plot) for each behaviour trait. The red line indicates the genome-wide
745 significance threshold and the blue dotted line indicates the suggestive threshold.

746

747

748 **Tables**

749 **Table 1.** Heritability estimates and standard deviations for behaviour traits using
750 pedigree and genotype data.

751 **Table 2.** Results for the genome-wide association study. Coordinates, statistics of
752 the REML analysis and positional candidate genes are given for all SNPs that
753 exceeded the suggestive or genome-wide significance threshold.

754 **Table 3.** Results for the regional heritability mapping. Coordinates, statistics of the
755 association analysis, regional heritabilities and positional candidate genes are given
756 for all genomic regions that exceeded the suggestive or genome-wide significance
757 threshold. Due to the sliding-window approach used in the analysis, the regions
758 comprise 50 SNPs and can overlap with adjacent regions by 25 SNPs.

759 **Supplementary Files**

760 **S1 Table.** Description of the behaviour traits used as phenotypes. Behaviour traits
761 were generated using a principal component analysis (PCA) on questions from the
762 C-BARQ questionnaire and additional questions about playfulness.

763 **S2 Table.** Lifestyle variables that were fitted as fixed factors in the statistical
764 analyses of behaviour traits. Description of lifestyle variables that were assessed
765 using the lifestyle survey (“Variables”) and individual models for every behaviour
766 trait where variables fitted as fixed effects in the models are indicated by “x”
767 (“Models”).

768 **S3 Figure.** Principal component analysis of the genomic data. Eigenvalues for the
769 first two principal components are plotted and individuals are coloured according
770 to their cohort (blue=UK or pink=Sweden).

771 **S4 Figure.** Q-Q plots and lambda values in parentheses for the genome-wide
772 association study of the 13 behaviour traits.

773 **S5 Figure.** Regional association plot. The $-\log(P)$ values calculated in the GWAS,
774 gene annotations and local linkage disequilibrium patterns are plotted for regions
775 identified by the regional heritability mapping that harbour genes. Neighbouring
776 and overlapping regions (due to the sliding-window approach) were plotted
777 together. The SNP with highest $-\log(P)$ from the GWAS is coloured in blue and all
778 others are coloured according to their r^2 to this SNP with white for no LD ($r^2 \leq 0.2$),
779 yellow for weak LD ($0.2 \leq r^2 < 0.5$), orange for moderate LD ($0.5 \leq r^2 < 0.8$) and red for
780 strong LD ($r^2 \geq 0.8$).